Lesions of the Dorsal Medial Hippocampus induce different forms of Repetitive Behaviour in the rat

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In the name of Allah, the Most Merciful, the Most Beneficient

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Abbreviations

DDG	Dorsal Dentate Gyrus
DG	Dentate Gyrus
METH	Methamphetamine
ARB	Abnormal Repetitive Behaviour
s.c	Subcutaneous
i.p	intraperitoneal injection
mg/kg	milligrams per kilograms (drug dosage)
µl	microlitre
mm	millimitre
h	hour
min	minute
AP	Anterior-posterior
ML	Midline
DV	Dorsal-ventral
SEM	Standard Error of Mean
ANOVA	Analysis of Variance
GLM	General Linear Model
SNK	Student Neuman-Keuls
DMH	Dorsal Medial Hippocampus

Abstract

The dorsal dentate gyrus (DDG) of the hippocampus plays a role in the expression of different forms of flexible behaviour mainly due to its ability to sustain neurogenesis throughout life. In the present thesis, we examined the role that the DDG and its adjacent areas, both collectively referred to as dorsal medial hippocampus (DMH), play in flexible, adaptive behaviour and cognitive processing. We used the neurotoxin, colchicine, to induce lesions of the DDG, which were found to affect neighbouring areas. Thus these lesions will be referred to as lesions of the DMH. In the first experiment, rats were tested for (1) perseverative behaviour before and after receiving chronic methamphetamine (METH) treatment, (2) METH-induced locomotor activity and stereotypy in an open field, and (3) working memory in a T-maze. The results showed that rats with lesions of the DMH exhibited perseveration and supersensitivity to the locomotor- and stereotypy-inducing effects of METH (0, 0.1, 0.3, 1 mg/kg i.p.) as well as increased long-term METH sensitization. Rats with DMH lesions also showed significant working memory deficits. Taken together, these results reveal specific forms of behavioural inflexibility in rats with lesions of the DMH that are mainly associated with perseveration, drug-related behaviours, including stimulant motor supersensitivity and drug sensitization, and impaired working memory functions.

Keywords: Methamphetamine, Colchicine, dorsal dentate gyrus, perseveration, locomotor activity, stereotypy, working memory.

Introduction

Repetitive behaviour

Both human and animal behaviour comprises of a wide range of repetitive behaviours. These are prominent behavioural manifestations that form a part of the normal behavioural repertoire. A diversity of repetitive behaviours has been observed in virtually all species of animals. For example, in invertebrates, birds and lower mammals, fixed, repeatedly performed action patterns are vital for the survival of both individuals and species, and in higher mammals, repetitive actions such as highly skilled acts acquired through practice, occur as a part of normal behaviour. However, abnormal repetitive behavior (ARB) also occurs in animals and can take numerous forms, from pacing (e.g., birds, prosimians, large carnivores), jumping and somersaulting (e.g., mice) to crib- and bar-biting (e.g., horses, pigs, mice), rocking (e.g., primates) and self-injurious behaviour (e.g., monkeys, parrots) (Langen, Durston, Kas, van, & Staal, 2011; Langen, Kas, Staal, van, & Durston, 2011). A range of ARBs spontaneously occur in humans in a variety of neurodevelopmental (e.g., Fragile X syndrome, Rett's syndrome), psychiatric (e.g., Obsessive–Compulsive Disorder, Impulse Control Disorders), and neurological disorders (e.g., Tourette syndrome, Parkinson's disease) (Lewis and Kim 2009).

Neural basis of repetitive behaviour

Researchers over the years have been increasingly intrigued by the neurobiological basis that may be responsible for the occurrence of ARB. This raises the question of whether these similar classes of behaviour are caused by similar, overlapping neurobiological mechanisms or instead they are the result of different neurobiological processes. Some researchers have previously tried to study the neuronal networks involved in the development and expression of repetitive behaviour.

In order to understand the neurobiological mechanisms of repetitive behaviour, animal studies are very useful. Traditionally, it was the basal ganglia which served as the main candidate for explaining repetitive behaviour. As early as the 1920s, the striatum was directly implicated in

studies of drug-induced repetitive behaviour in guinea pigs (Amsler, 1923) and since then, many studies have used diverse techniques to confirm that damage to the basal ganglia results in 'recurrent perseveration' or inappropriate response repetition (Garner, 2005; Norman and Shallice, 1986; Sandson and Albert, 1984; Turner, 1997). Many early studies focused on the development of repetitive motor behaviour and largely ignored striatal influences on other, non-motor repetitive behaviour. However, accumulating evidence led to a challenge of this view and in a pivotal paper in 1986, Alexander and colleagues dramatically redirected basal ganglia theory and research (Alexander et al., 1986). They reviewed earlier ideas and studies of basal ganglia function (e.g. DeLong et al., 1984; Kunzle, 1975, 1977, 1978; Nauta, 1979; Schell and Strick, 1984) and proposed that the basal ganglia should be viewed as components of multiple parallel, segregated circuits with outputs targeting not only primary motor areas, but also specific pre-motor and prefrontal cortical areas. Thus, Alexander and colleagues (1986) suggested *five parallel corticostriatal circuits*. They referred to these circuits as: (1) the motor circuit, (2) the occulomotor circuit, (3) the dorsolateral prefrontal circuit, (4) the lateral orbitofrontal circuit, and (5) the anterior cingulate circuit. Later, Middleton and Strick (2000) described two additional circuits between the basal ganglia and more posterior parts of the cortex (the inferotemporal and posterior parietal circuits). Each circuit was proposed to include discrete, essentially non-overlapping parts of the striatum (caudate nucleus, putamen and nucleus accumbens), globus pallidus, substantia nigra, thalamus and cortex. Circuits are structured (Figure 1), with each circuit receiving cortical inputs to the striatum, passing the input through the basal ganglia, via output nuclei (the substantia nigra pars reticulata and the medial globus pallidus) to a restricted area of the thalamus and from there back to a single cortical area (Ring and Serra-Mestres, 2002). Furthermore, each loop consists of *two distinct branches:* the direct (or striato-nigral) and the indirect (or striato-pallidal) pathway. The net result of activity of the direct pathway is an increase in thalamic activity, whereas activity of the indirect pathway inhibits the thalamus. Thus, under normal circumstances, the direct pathway enhances behaviour, whereas the indirect pathway inhibits it (Lewis et al., 2006). This dual system is considered to allow for fine-tuning of activity in large portions of frontal cortex responsible for movement, cognitive and limbic functions (Bradshaw, 2001).

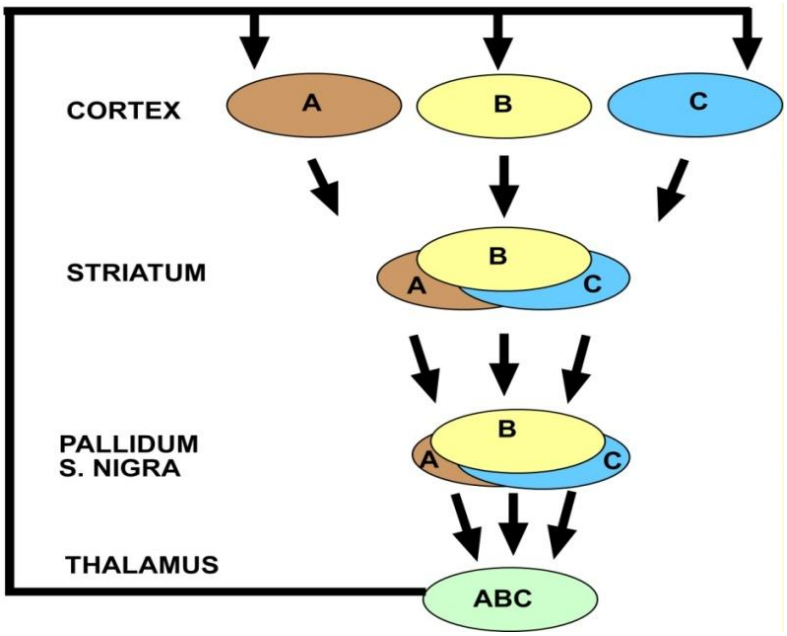

Figure 1.Corticostriatal circuits as proposed by Alexander et al. (1986). Each circuit receives output from several functionally related cortical areas (A, B and C) that send partially overlapping projections to restricted parts of striatum. These striatal regions send converging projections to the globus pallidus and substantia nigra, which in turn project to specific regions of the thalamus. Each thalamic region projects back to one of the cortical areas that feed into the circuit, thereby completing the ''closed loop''.

It is now established that cortico-striatal loops can be functionally divided into three *'macro-circuits'*, related to the predominant cerebral cortical input to striatum. These are the sensorimotor circuit (comprising the motor and oculomotor loops), the associative circuit (dorsolateral prefrontal loop) and the limbic circuit (lateral orbitofrontal and anterior cingulate loops (Groenewegen et al., 2003). Within these macrocircuits, smaller (micro)-circuits can be recognized that subserve specific functions within the broader functional domain, i.e., sensorimotor (movements), associative (cognitive functions) or limbic (emotional–motivational behaviour) (Groenewegen et al., 2003; Mason and Rushen, 2006). In the original description of the five parallel circuits, Alexander described how damage to individual loops may lead to ARB. He implicated the orbitofrontal circuit in behavioural inhibition and switching behaviour, as studies in primates had shown that bilateral lesions to the lateral orbitofrontal area or to the

portion of the caudate to which it projects result in perseverative behaviour (Alexander et al., 1986). With the functional integration of the corticostriatal loops, it is recognized that repetitive behaviour may reflect a disruption of coordinated function within the basal ganglia or between striatal and forebrain structures (Robbins et al., 1990). As such, ARB may result from damage to any of the circuits and the exact location of the disruption (i.e., which loop is affected) may determine what type of repetitive behaviour is displayed (Mason, 2006).

Apart from the basal ganglia, other important structures that play a key role in the development and expression of ARBs are the frontal cortex and hippocampus. Disruption in the frontal-striatal pathways lead to difficulties inhibiting unwanted movements (stereotypy), inability to change mental set in the form of perseveration or compulsive behaviour patterns that form important core components of repetitive behavior (Langen et al 2011). Attention, along with planning skills, is compromised due to frontal cortex pathology. Inability to adapt to new situations or carry out complex, purposive and goal-directed activities coupled with restlessness and impulsiveness are common markers in repetitive behaviour. These functional abnormalities are the resultant effects of frontal lobe abnormalities. Since the frontal cortex has important interconnections with the striatum and basal ganglia, functional anomalies in movement and higher-order cognitive processes are all affected in conjunction.

Neurobiology of Repetitive behaviour: Role of Hippocampus

An emerging view of the hippocampus is that of a functionally heterogeneous structure along its longitudinal axis. Differences in functionality have been observed across dorsal and ventral parts of the hippocampus. Lesion studies reveal that the dorsal (septal pole) hippocampus is involved in learning and spatial memory, whereas the ventral (temporal pole) hippocampus regulates emotional and motivated behaviours (Fanselow and Dong, 2010). This functional dissociation has been supported by anatomical connectivity and gene expression analyses. For example, serotonergic fibers provide denser input to the ventral hippocampus with a concomitant enrichment of 5-HT1A and 2C receptors ventrally (Tanaka et.al. 2012). Efferent

connectivity studies indicate that ventral hippocampus can modulate reward circuitry and emotional behaviour through projections to nucleus accumbens, prefrontal cortex and amygdala, and stress responses by regulating the hypothalamic– pituitary–adrenal axis (Sahay and Hen, 2007). Evidence accumulated suggests that the hippocampal formation modulates basal ganglia activity by means of extensive, topographically-organized projections to the striatum (Groenewegen et al., 2003), and may therefore be in a position to influence the expression of repetitive patterns of behaviour mediated by the basal ganglia. Some lesion studies indicate that damage to the hippocampus alters a range of action patterns and behaviours including stimulant-induced motor behaviour (Schaub, Schmelzeis, Mittleman, 1997), stimulus-response learning (Sanderson et al. 2012) and impulsive choice (Mariano et al. 2009). The hippocampus sustains adult neurogenesis, one of the most remarkable forms of physiological plasticity and functional modifiability in the brain. Deficient neurogenesis has been linked to a range of processes including increased sensitivity to drug-induced reward and motivation to seek drug reinforcement, enhanced HPA axis responsiveness, emergence of withdrawal-induced negative states, such as anhedonia, anxiety and depression, wide-ranging memory impairments, and potentiation of inflexible behaviour, including impaired extinction learning and enhanced habit learning (Canales, 2012). The aim of the present research is to study whether lesions of the dorsal dentate gyrus (DDG) affect repetitive patterns of cognitive processing and behaviour in rats.

Neuropsychiatric disorders and repetitive behaviour

In modern-day neuropsychiatry, the term repetitive behaviour is an umbrella term, used to refer to broad and often disparate classes of behaviour linked by repetition, rigidity, invariance, and inappropriateness and observed in a wide array of developmental, psychiatric and neurological disorders (Turner, 1999). Repetitive behaviours are the core syndrome of numerous neurological and psychiatric disorders such as obsessive-compulsive disorders, Gilles de la Tourette syndrome and autistic spectrum disorders. They are also a feature of substance drug addiction including dopamine dysregulation syndrome in Parkinson disease, behavioural

addictions such as pathological gambling or compulsive eating and traumatic or surgical lesions of the frontal lobes. Studies investigating stereotypies induced by drugs of abuse have shown an excess brain dopamine function which has been linked to stereotypies found in psychotic disorders (Robinson and Becker 1986), stereotypies observed in persons with mental retardation (Bodfish et. al 1996) and dyskinesias associated with L-dopa therapy in Parkinson disease (Graybiel et. al 2000). There are, however, striking similarities between the wide variety of ritualistic, repetitive, stereotyped and compulsive behaviour observed in normal individuals and the ritualistic and compulsive behaviour observed in these psychiatric disorders. Across the wide spectrum of disorders, many forms of behaviour are included in this term of repetitive behaviour, which includes stereotypies, rituals, obsessive and compulsive behaviour, circumscribed interests, echolalia, tics, perseveration and self-stimulation or self-injury. Across such wide range of neuropsychiatric disorders with multifaceted symptomatology, the core behaviour pattern is *ARB*. ARB is observed across different species and has been found to have multiple manifestations, ranging from basic motor behaviour to higher level cognitive functions.

Drug-induced locomotor activity and stereotypy as a form of repetitive behaviour

Repetitive behaviour affects motor functions in the form of various motor tics and can be elicited by stimulant drugs. Stimulant-induced behavioural stereotypies are characterized by relatively enduring, apparently purposeless and repetitive movements in rats. Schiorring (1971) refers to the behaviour as “stereotypy phase”. They observed that stereotypies occur at doses above those that induce locomotion (Rebec et al. 1997) and below those that result in self-mutilation (Creese and Iversen, 1972).

Pathological repetitive behaviour in the form of abnormal motor responses may include a wide range of behaviours, ranging from hyperactivity to stereotypy. Various studies have been done on animals to find out the relationship between neurogenesis and ARB with motor manifestations. Jens Malmkvist, Bjarke Brix, Kim Henningsen and OveWiborg (2012) studied adult hippocampal neurogenesis in minks. Their study finding however did not confirm the

hypothesis that abnormal behaviour is linked to reduced hippocampal neurogenesis. Instead, they found a positive correlation between cell proliferation and stereotypic behaviour in minks. Hippocampal neurogenesis increased with increasing performance of stereotypic behaviour and active/locomotor activity. Another study (Pytte, Gerson, Miller, Kirn, 2007) was conducted to examine the relationship between adult hippocampal neurogenesis and stereotypic song structure in Zebra finch birds. Their study findings revealed that between 4-15 months of age, there was an increase in the stereotypy of fine-grained spectral and temporal features of syllable acoustic structure in Zebra finch. They also found that over the same age-range there was a decrease in the addition of new neurons in the hippocampus. These seemingly contradictory findings indicate that a better understanding of the possible role of hippocampal neurogenesis in the expression of ARB is needed.

Repetitive Behaviour and Lesions

We worked with the hypothesis that the DDG of the hippocampus is implicated in flexible behaviour. Perseverative behaviour and drug-induced stereotypy are two forms of behavioural inflexibility that can be studied in experimental animals. We expected lesions of the DDG to produce deleterious effects on perseveration and stereotypy. Rats received discrete neurotoxic lesions of the dentate gyrus (DG) and were tested in standard behavioural tasks designed to measure such behavioural and cognitive parameters. The experiments performed throw light into the role of the DG of the dorsal hippocampus in behavioural flexibility.

Aims and Objectives

- To study the effect of bilateral DDG lesions on perseveration in a T-maze gambling task

- To study the effect of bilateral DDG lesions on methamphetamine (METH)-induced locomotor activity

- To study the role of bilateral DDG lesions on sensitization to METH

- To study the effect of bilateral DDG lesions on perseveration in a T-maze gambling task after administration of METH

- To study the effect of bilateral DDG lesions on working memory function

Methodology

3.0 Experimental Materials and Methods

The work described in this thesis is based on the results of neurotoxic lesions of the DDG, a method that over the past few decades has been widely used to elucidate the role of discrete functions of the brain. We used Watson and Paxinos' rat brain coordinates to locate the specific areas of the hippocampal DDG of the brain to be lesioned.

Behavioural Experiments

All experimental rats received colchinine-induced lesions in the DDG while the control rats received sham lesions. The ensuing sections provide a summary of the various surgical, experimental, behavioural, histological and statistical procedures that were utilized during the present investigation. All procedures were approved by the University of Canterbury, Animal Ethics Committee (Application no. 2013/03R).

3.1 Animals and surgery

Male Long Evans rats, weighing (250-300 g) at arrival in the lab, were bred in the Department of Psychology, University of Canterbury. In all cases, they were handled extensively for four days before surgery. They were initially housed in groups of four under constant conditions of temperature (19-21^{o}C), relative humidity (60-65%) and light dark cycle (12:12 hrs, lights on at 8:00 pm) with standard lab rat chow and tap water available ad libitum. 20 animals (10 experimental and 10 control) were used for the first behavioural experiment. All rats weighed 250-300 g at the time of surgery. Lesion surgery was based on the protocol utilized by Hernandez-Rabaza et al. 2007 and 2008. All animals received injections of cephalexin (s.c) one day before surgery as well on the same day, before surgery. One hour before surgery, the rats

were given 5 mg/kg of carprofen to induce analgesia. The same treatment was repeated 8 h after if any sign of distress (e.g., hunched posture, inactivity) was evident in the animal. Following carprofen treatment, the animals were anaesthetized with Avertin, an injection prepared from 2,2,2,-tribromoethanol (12.5 mg/ml solution, dissolved into 2.5% tertiary amyl alcohol, dose of 250 mg/kg i.p.) and mounted on a stereotaxic apparatus (Stoelting, Illinois) on a flat skull position. Clippers were used to shave the head and the skin was cleansed with antiseptic solution of betadine and alcohol.

The skull was exposed and an inverted V-shape hole was drilled in the skull at the level of the DDG of the hippocampus, bilaterally. A stainless steel needle (31G) was mounted on the stereotaxic arm and connected with polyethylene tubing to Hamilton microsyringe driven by a precision pump (Harvard Apparatus). A quantity of air equivalent to approximately 0.2µl of fluid, as measured by the microsyringe, was then pulled into the system. Immediately after, a sufficient quantity of colchicine was drawn in and the system was tested. There are 2 ways to check if infusion into the brain was successful:

1. The air bubble separating the fluids in the system could be observed to slowly move downwards during the infusion.
2. The system was tested immediately after retrieval of the injectors from the brain to verify their patency.

However, it must be noted here that none of these methods warranted absolute certainty of drug delivery, but they provided a good indication that the infusion was effectively made into the brain. The microinjections infused into the brain were always bilateral into the DDG and the volume of colchicine infused was kept constant at 0.2µl in each site. The infusion rate for all lesion surgeries was 1.0 µl/min and the diameter of the microsyringe set at 0.343 mm in the precision pump.

Lesion rats received a total of 10 injections (0.2µl each) of the neurotoxic solution (colchicine), five into each hemisphere. The sham rats received the same amount of saline in each of the brain sites bilaterally. The needle was carefully lowered into the brain at the following stereotaxic coordinates: AP −2.3, −3.1, −3.8, −4.5, −5.3, ML ±0.7, ±1.10, ±1.80, ±2.4, ±3.2, DV 3.6, −3.7, −3.7, −3.1, −3.2 (from brain surface). The needle remained in place for 2 min to reduce backflow. The wound was cleansed and Neotopic H Lotion was applied (the lotion contains antibiotic and local anaesthetic). Sutures were applied as needed.

During surgery the animals were kept warm with an electric blanket. The rats were allowed to recover from the surgery for 7 days in the recovery room. Rats were monitored twice a day, including weights, feeding patterns and general motor behaviour.

3.2 Food deprivation schedule

During the second week of recovery (8 days after the surgery) all rats were subjected to the food deprivation regime. The main purpose of the food deprivation schedule was to motivate the rats to perform tasks for food where food acted as the primary reinforcer. Deprivation was to 90% of initial body weight (this was the weight of the animal after 7 days recovery from surgery) with food rationed accordingly. Animals were weighed three times a week to ensure they did not fall below 90%. The behavioural experiments commenced two weeks after the surgery.

3.3 Experiment 1: Gambling task performance of lesioned and sham rats.

Research question: Does bilateral DDG lesions increase perseveration in a gambling task before administration of METH?

Specific objective: Two weeks after the surgery, rats were food deprived and kept at 90% of their free feeding weight. Rats were placed in a T-maze to perform a guessing task (bias-corrected two choice gambling test of Garner et al., 2003). Essentially the test consists of guessing the correct location of a food reward out of two possibilities (left or right side of the T-maze). There is guessing involved because the position of the food reward is determined pseudo-randomly. The task measures perseveration since it can detect repetitive patterns of choice.

Rats were habituated to the maze for 30 min, allowing free exploration of the apparatus. Food pellets were scattered throughout the maze. Once the rat ate the pellets, or 10 min elapsed, whichever occurred sooner, the rat was returned to its home cage. On the next day, a shaping phase began. In this phase, food was presented in the food cup at the end of the left and right arms. This was repeated three times. The next stage was the training phase (experiment phase) where 100 trials were given to assess perseveration. The rat began at the start box. The reward cup was determined at random with a probability equal to the observed probability of choosing the opposite cup in the previous 20 trials (the first 20 trials are completely random). One bowl was baited out of sight of the rat. The trial started when the dividing guillotine door was lifted. The first bowl that the rat approached was designated as the choice for that trial. If the rat's choice was unsuccessful, it was not allowed to collect reward from the other bowl. The rat was then returned to the start box. Each rat performed 50 trials per day.

Statistical analysis: Initial data processing was performed automatically by the gambling task software (Garner et.al 2003; Garner et.al 2011). Perseveration on this task was quantified in a variety of ways. When significant side bias was present, Markov analysis allowed the sequence

to be quantified for non-randomness corrected for side bias (Garner et.al 2003). Perseveration was quantified as the number of trials where the previous response was repeated. Accordingly we also quantified response latencies for both response types. Finally side bias (the overall chance of choosing one side or another) captured the sensitivity of animals to reward contingencies in general. Accordingly side bias was calculated as the overall deviation from perfect unbiased 50/50 choice.

The number of perseverative choices was analyzed as a repeated-measures Poisson regression using PROC GENMOD in SAS 9.4 for Windows. Treatment (Lesion *versus* Sham), Timepoint (Pre *versus* Post METH exposure), and their interaction were tested, with repeated measures taken on Subject nested within Treatment. Poisson regression is ideally suited for such Poisson-distributed count data (Littell et. al 2002), and provided a better model fit over a Mixed Model (which assumes a normal distribution). Effects were tested with Type III Likelihood Ratios (equivalent to Type III sums of squares in a GLM or ANOVA framework). The resulting Mean counts +/- SEM were graphed as percentage of responses for clarity.

Response latencies typically followed a log-normal distribution, and accordingly were logged before being averaged for each individual, following previous publications using this task (e.g. (Garner et.al 2003). The resulting Mean log latencies followed a normal distribution and were therefore analyzed as a REML Mixed Model in JMP Pro 10 for Windows. The same model was tested (i.e. Treatment, Timepoint, and their interaction were tested, as repeated measures on Subject nested within Treatment).

Side bias was expressed as a count of choices deviating from 50/50, and thus the same Repeated Measures Poisson Regression analysis employed as for perseverative choices. Post hoc Tukey tests were used to examine differences between means.

3.4.Experiment 2: Acute locomotor activity in lesioned and sham rats

Research question: Does bilateral DDG lesions alter METH-induced locomotor activity?

Following one week of food maintenance, rats were exposed to an open field (50 x 50 cm) for 1 hour in a drug-free state (habituation) and then returned back to their home cage. Treatments began the next day. Each group received METH at doses of 0, 0.1, 0.3 and 1 mg/kg i.p. in a counterbalanced fashion and locomotor activity patterns were monitored for 1 hour. Each of these treatments was administered on a different day.

Statistical analysis: Analysis of Variance (ANOVA) was computed using Statview program to find out the main and the interaction effects of the lesion and the locomotor activity recorded in terms of the distance travelled by the animals in the open field chambers. Interaction effects were analyzed by the method of Newman-Keuls.

3.5 Experiment 3: Sensitization to METH in lesion and sham rats.

Research question: Does bilateral DDG lesions increase sensitization to METH?

Specific objective: After the acute locomotor activity experiment, all rats were sensitized to METH. In this test, rats received 2 mg/kg of METH for 7 consecutive days. Stereotyped behaviour was measured daily with a rating scale. Four parameters of stereotyped behavior were assessed, namely repetition, frequency, duration and spatial distribution (Canales and Graybiel, 2000). *Repetition* incorporated the reiteration of a particular stereotyped behaviour; *frequency*, the number of times a particular stereotyped behaviour was displayed; *duration*, the length of time engaged in the performance of a stereotyped behaviour and *spatial distribution* referred to the extension of the physical area where the performance of the stereotyped

behaviour took place. All four parameters were measured twice during the 60 min session. The first assessment was done 20min from the start of the experiment and the second after 50 min from the onset of the experiment. The sensitization phase was followed by an abstinence phase of 3 days where rats were not exposed to the open field chambers and no drug was administered.

Behavioural Analysis: Behavioural responses were monitored systematically after acute injections of METH. A time-sampling procedure based on the Creese and Iversen stereotypy scale (Creese and Iversen, 1972) was used to score motor stereotypies. Each animal was observed twice for 1 min, at 20 min and 50 min after the injection, and the average of the 2 scores was considered as the overall session score. The observer was blind to the experimental conditions. Scores were based on estimates of four behavioural dimensions: the number of alternative motor responses emitted (repetitiveness/flexibility),the number of responses per unit time (frequency), the percentage of time spent performing the most dominant responses (duration)and the level of spatial confinement of the motor response (spatial distribution).Motor responses examined included head (head swaying, head bobbing) and limb (forelimb, hind limb) movements, stereotyped sniffing, grooming and rearing and oral stereotypies (licking, nibbling). The behavioural analysis was performed in the same way as was done by Canales and Graybiel (2000).

Statistical analysis: Statview program was used to compute ANOVA to examine the main and the interaction effects of the lesion and the sensitization behaviour recorded in terms of the distance travelled by the animals in the open field chambers. Post hoc test of Neuman-Keuls was done using Statview.

3.6 Experiment 4: Locomotor activity in a sensitization probe test (pre METH and post-METH) in two groups of rats.

Research question: Does bilateral DDG lesions increase locomotor activity after administration of METH?

Specific objective: The 4th experiment immediately started after the abstinence phase. The 3-day abstinence phase was followed by a probe (0.3 mg/kg dose of METH) administered to both groups of rats. This low dose tested for sensitization to METH. Withdrawal from the semi-chronic METH treatment did not generate strong physical signs. Rats were monitored twice daily and we recorded weight, general activity (e.g., locomotion, grooming) and appearance of the fur.

Statistical analysis: Statview program was used to compute ANOVA to assess the main and the interaction effects of the lesion and the probe test, i.e., comparison between the pre-METH and the post-METH treatment conditions. Neuman-Keuls post hoc analysis was computed to study the pairwise comparisons between group means.

3.7 Experiment 5: Post-METH gambling task performance in two groups of rats.

Research question: Does bilateral DDG lesions increase perseveration in a gambling task after administration of METH?

Specific objective: 13 rats (8 lesioned and 5 shams) were exposed to the T-maze to run the bias-corrected two choice gambling task again. 3 animals (1 lesioned and 2 sham rats) died in home cages and 4 rats (1 lesioned and 3 shams) were removed from the study as data could not be collected properly due to video-tracking problems. Since the rats received METH treatments in the probe test, this test assessed their post-METH performance on the perseveration task. No habituation was given as the rats were familiar with the T maze. Data was collected in a similar

fashion as was the baseline data where each rat performed 50 trials per day. The probability of baiting the right or left food arm was randomly generated by the program. All data was recorded in the Microsoft access program.

Statistical analysis: PROC GENMOD in SAS 9.4 for Windows was used to analyze the number of perseverative choices as a repeated-measures Poisson regression. Post hoc Tukey tests were used to examine differences between means.

3.8. Experiment 6: Delayed alternation test performance in lesion and sham rats.

Research question: Does bilateral DDG lesions affect working memory function?

Specific Objective: A delayed alternation task followed the guessing task in the T maze where all rats were trained to run the maze. Each rat performed 10 trials per day. Food was randomly baited in each trial with food placed in the right arm in 5 trials and another 5 trials in the left. In the 1^{st} trial, or sample trial, if food was baited in the right arm then the rat was allowed to run up to the right arm and eat the pellet. Conversely, if food was placed in the left arm then rat was forced to run to the left and eat the food. In the next trial, or test trial, the rat was given free access to both arms and food was placed in the opposite arm. Correct choices in the test trials were recorded. In this way each rat performed 10 trials for 7 days. All data was recorded.

Statistical analysis: ANOVA was calculated to find out the differences between means in the treatment groups and performance on the delayed alternation task.

3.9. Perfusion of the brain for preparation of histology

On completion of the behavioural experiments, rats were deeply anaesthetised with pentobarbital (125 mg/kg) and perfused transcardially to fix (i.e., using 4% paraformaldehyde in 0.1 M phosphate buffer) and remove the brain, which was processed for immunocytochemistry. During this procedure, once the rat was under deep anaesthesia (i.e., totally unconscious and insensitive to pain as determined by tail and plantar reflexes) we opened up the thoracic cavity to expose the heart, we then inserted and secured a needle into the left ventricle and we started perfusing a heparinised saline solution. The death of the animal actually occurs during exsanguination. The exsanguination was achieved by making a small cut in the atrial region of the heart at the time of inserting and securing the perfusion needle into the heart. Thus saline and fixatives entered the left ventricle and irrigated the brain, forcing the blood out through the atrium. This method was preferred to killing the animal before perfusion because it helped the process of complete blood removal from the brain. This is important because blood is highly immunoreactive and interferes with histological techniques. Following saline perfusion, we then perfused using the fixative and removed the brain.

3.10. Histological findings

In the majority of lesioned rats (9/10), the lesions extend from -1.88 to -5.60 mm posterior to bregma. The DG dorsal CA1 and medial subiculum were damaged in all the lesioned animals. Partial CA2 and complete CA3 were relatively spared in all the animals [Figure 2]. 2 rats had unilateral DG lesion along the antero-posterior extent of the hippocampus [Table 1]. Cortical thinning was found in 3 animals. At -5.60 mm posterior to bregma, 2 animals had partial bilateral DG loss while at -6.04 mm posterior to bregma, one rat had unilateral DG loss. The most ventral parts of the hippocampus were spared in all the animals [Figure 3].

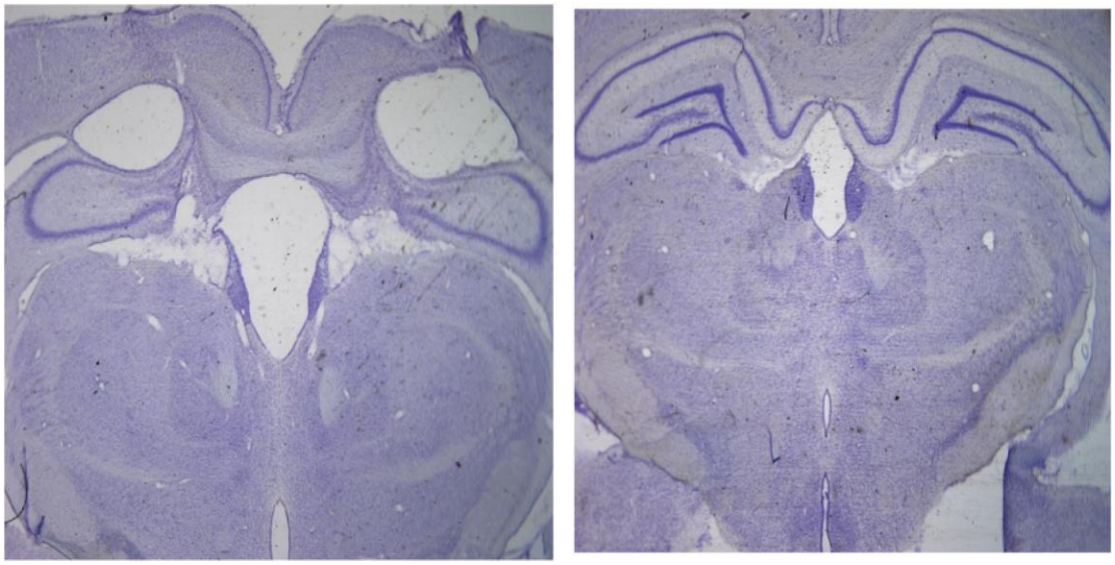

Figure 2. An example of coronal sections of a lesioned animal with the extent of damage (left) and an intact hippocampus in a sham-operated rat (right).

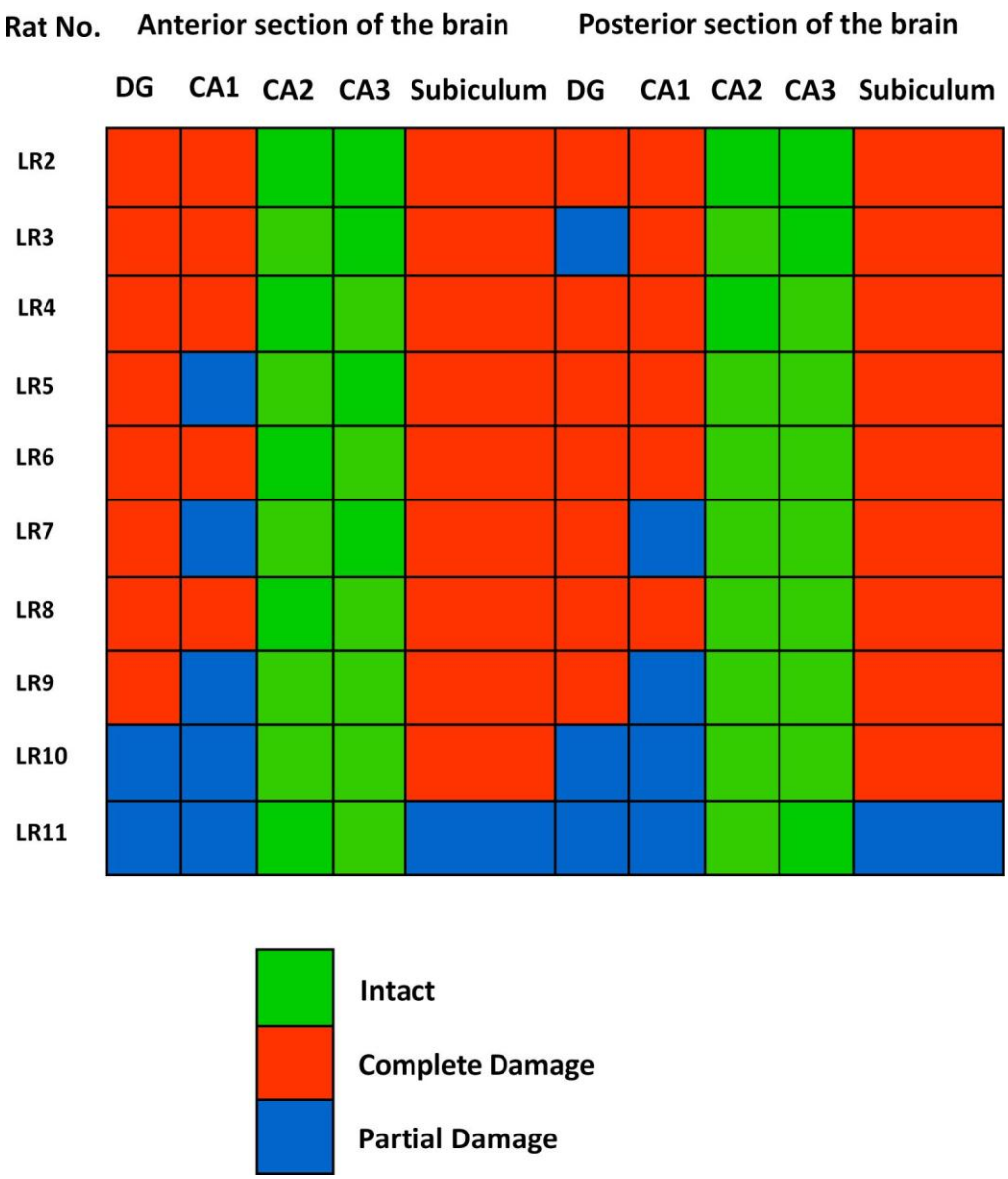

Table 1. A comparative chart showing the affected and unaffected areas of the brain caused by the colchinine-induced lesion in rats.

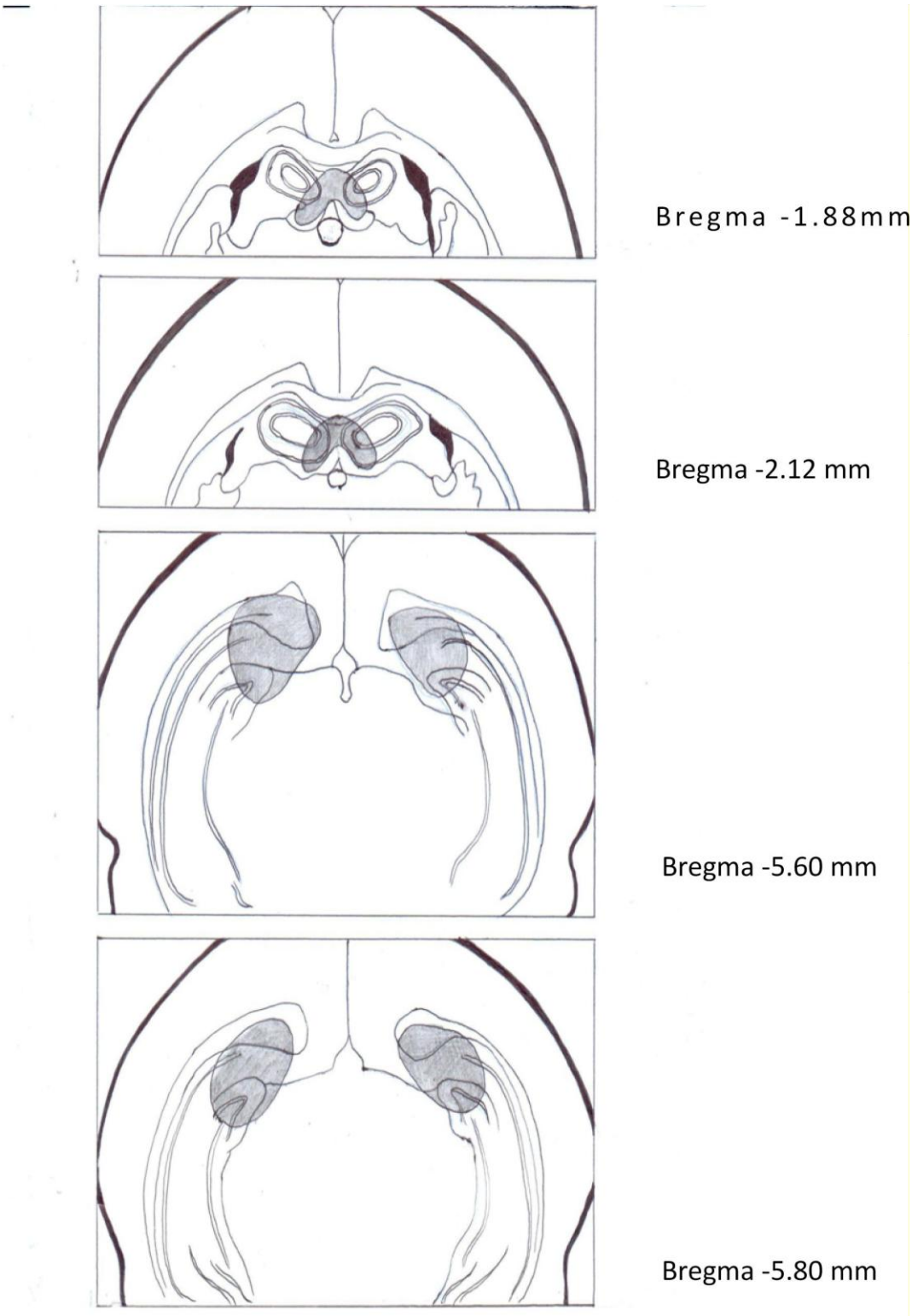

Figure 3. Schematic reconstruction of the dorsal hippocampal lesions. Coordinates of the brain sections are indicated with reference to bregma according to the stereotaxic atlas of Watson & Paxinos (1986).

Results

4.1 Effects of lesion on perseveration

Rats were evaluated across several domains of repetitive behaviour, including before-after METH-induced perseveration, locomotor activity, sensitization to METH, METH-induced stereotypic behaviour and working memory functions, in order to study the effect of bilateral DDG lesions.

4.1.1 Perseveration

Work in animal models has implicated increased perseverative behaviour as a form of ARB. In the present study, rats were evaluated for perseveration in a T-maze gambling task (Figures 4-6). Lesioned animals made significantly more perseverative choices (Treatment LR Chi-Square = 10.41; P = 0.0013). Perseveration did not differ significantly following METH exposure (Timepoint LR Chi-Square < 0.01; P = 0.9765), and the effect of treatment was not significantly modulated by METH (Timepoint x Treatment LR Chi-Square = 0.80; P = 0.3724) [Figure 4].

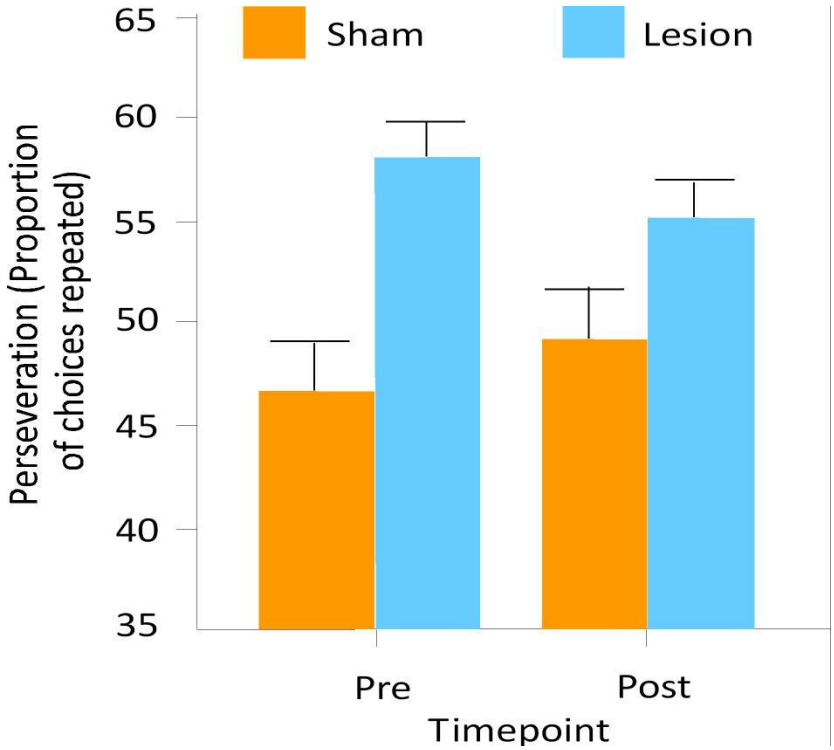

Figure 4. Lesioned animals showed significantly greater levels of perseveration at both timepoints. Bars represent means ± SEM.

4.1.2 Latency to choose

METH exposure significantly reduced the latency to make repeated ($F_{1,11}$ = 78.3494; P < 0.0001) and switched choices ($F_{1,11}$ = 80.5272; P < 0.0001). Lesioned animals did not show an increase in repeated ($F_{1,11}$ = 2.1988; P = 0.1662) or switched ($F_{1,11}$ = 3.0285; P = 0.1097) choice latencies; and the Treatment x Timepoint interaction was not significant for repeated ($F_{1,11}$ = 0.0057; P = 0.9412)or switched choices($F_{1,11}$ = 0.2829; P = 0.6054) [Figure 5].

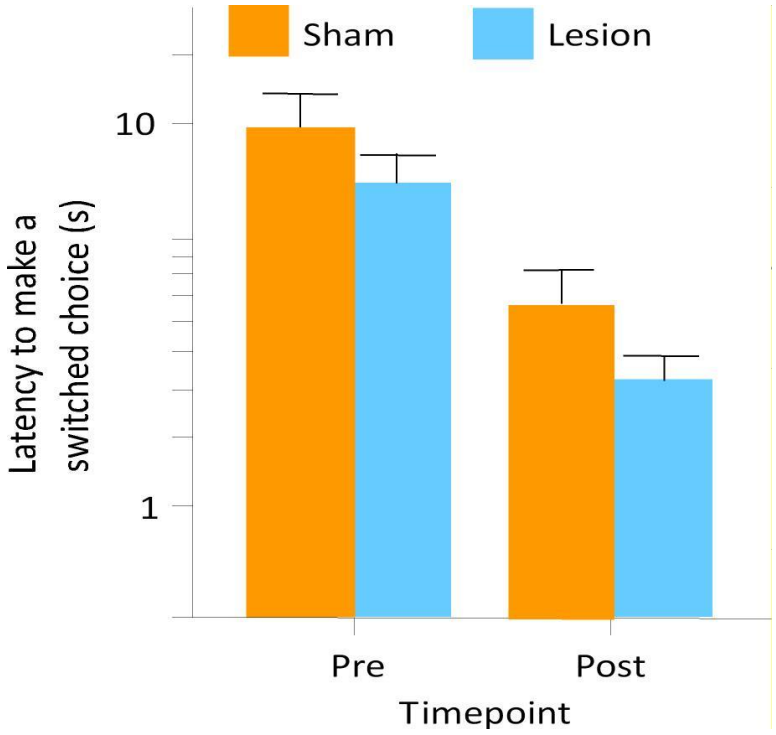

Figure 5: Methamphetamine treatment significantly reduced choice latencies for both repeated and switched choices. Bars represent means ± SEM.

4.1.3 Side Bias

Side bias showed a significant interaction between Treatment and Timepoint (LR Chi-Square = 4.72; P = 0.0299). Post Hoc Tukey tests revealed that this was due to a significant decrease in side bias in lesioned animals following METH exposure; whereas lesion and sham animals did

not differ prior to METH exposure; and sham animals did not show a significant change in side bias following METH exposure [Figure 6].

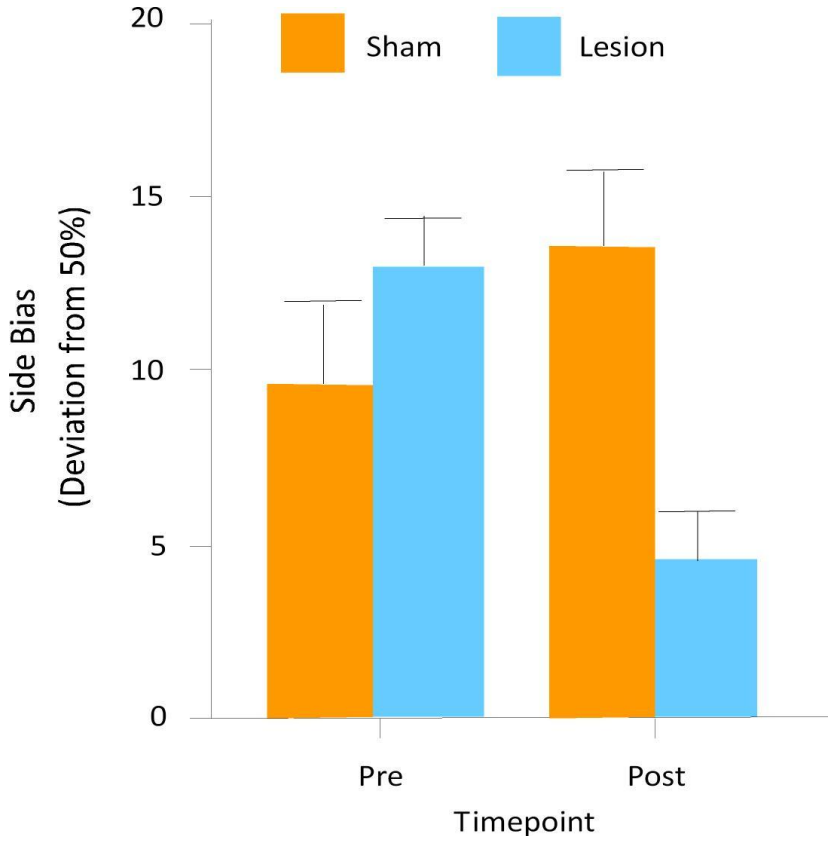

Figure 6: METH selectively reduced side bias in lesioned animals only. Bars represent means ± SEM.

4.2. Effects of lesion on acute locomotor activity

To determine whether the bilateral DDG lesions caused any alterations in METH-induced repetitive behaviour, locomotor activity was monitored. Analysis of the total distance travelled after the habituation period included all rats that received doses of 0, 0.1, 0.3 and 1 mg/kg of METH injections in a counterbalanced form. The main effect of the lesion ($F= 3.087$, $df= 1$, $p=0.09$) was not significant. However the main effect of treatment was significant ($F= 61.536$, $df= 3$, $P<0.0001$) and a significant interaction effect of lesions and METH was found ($F=6.851$, $df= 3$, $p=0.0006$). This indicated that the locomotor activity induced by METH in rats with lesions was increased as compared to sham-treated controls [Figure 7]. METH-treated animals

exhibited hyperlocomotion in the 1.0 mg/kg dose during the initial exploratory phases (i.e., the first 30 min) of the 2h session, compared to the 0.1 mg/kg dose.

Post hoc Student Neuman-Keuls (SNK) test was computed to investigate the complex comparisons between group means of the main effects of METH and time. The SNK post-hoc test revealed that the mean difference for METH doses of 0 and 1.0 mg/kg had a significantly higher mean difference value than for doses of 0.1 and 0.3 mg/kg ($p<0.05$).

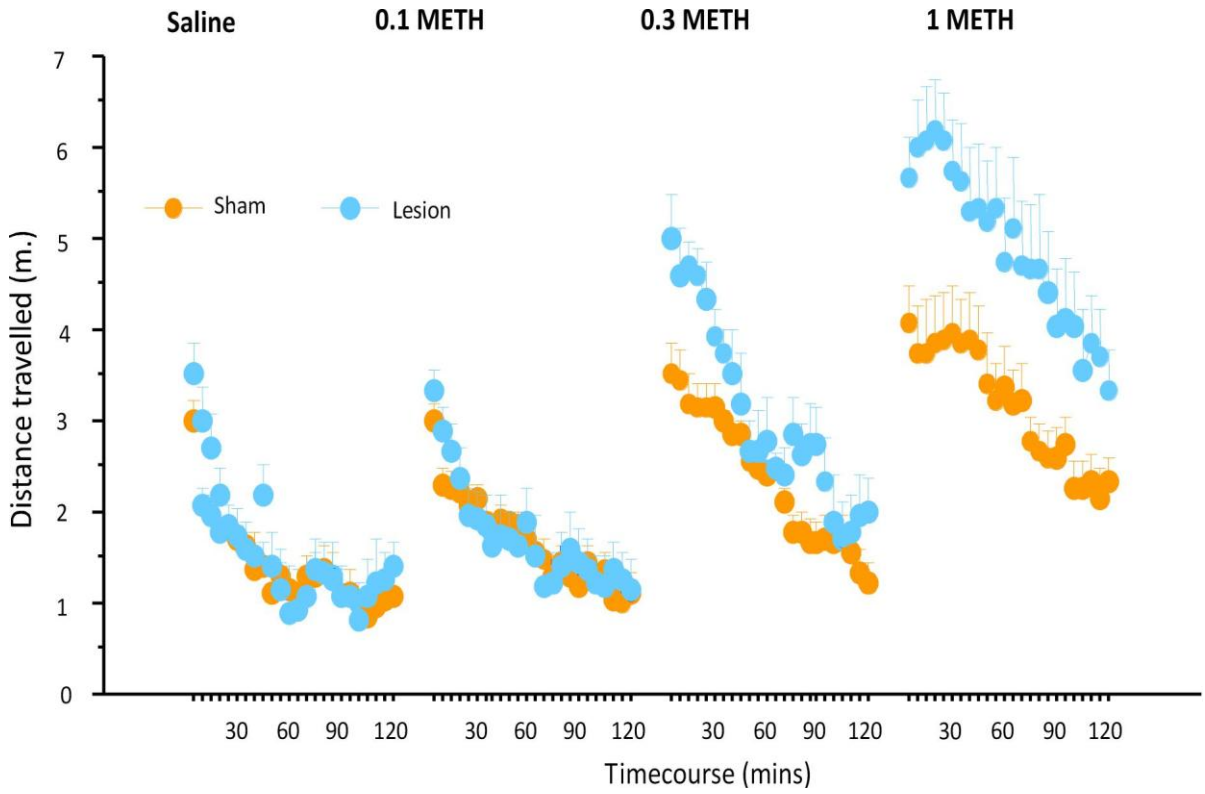

Figure7.Comparison of locomotor activity recorded as distance travelled (in meters) in four sessions (120 mins each) for saline and three different doses of METH i.p injections in lesioned and sham rats. Data points represent means ± SEM.

4.3. Effects of lesion on METH sensitization

To determine whether receiving a chronic neurotoxic regimen of METH produced any sensitization effects, all animals were administered a dose of METH (i.e., 2.0 mg/kg) for 7 consecutive days. Experimental animals displayed an increased sensitization to METH and

hyperlocomotion in the early phase of the session, as compared to the shams. The main effects of lesion (F= 1.063, df= 1, p=0.3229) was not significant. However, there was a significant effect of time on sensitization to METH (F= 3.366, df= 11, p= 0.0004). Also, the effects of time and lesion had a significant effect on METH sensitization (F= 2.463, df= 11, p= 0.0078) [Figure 8]. Additionally, the mean difference for time intervals of 5 min and 50 min resulted in significantly higher mean difference than for time intervals of 5 min and 10 min (SNK, $p < 0.05$). However, the effect of session and lesion was not found to be significant (F 0.432, df= 6, p=0.8550) [Figure 9].

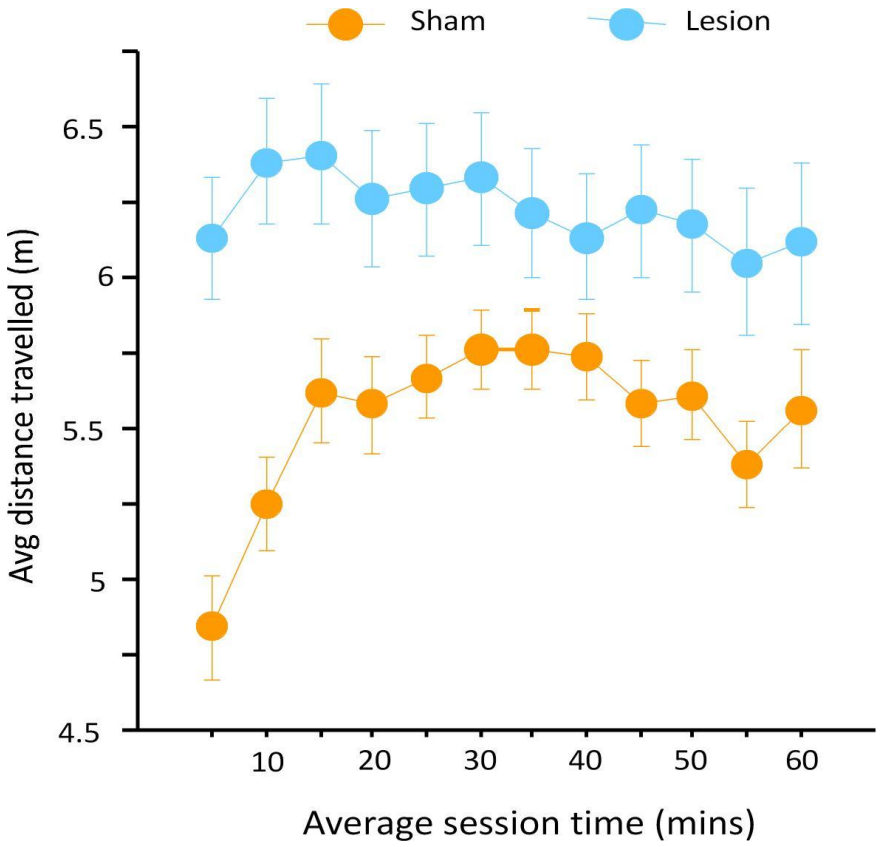

Figure 8. Average session time taken (in min) and average distance travelled by lesioned and sham rats in a 7 days treatment regimen of METH sensitization. Early in the session lesion rats appear to be more hyperactive. The graph shows an effect of time and lesion. Data points represent means ± SEM.

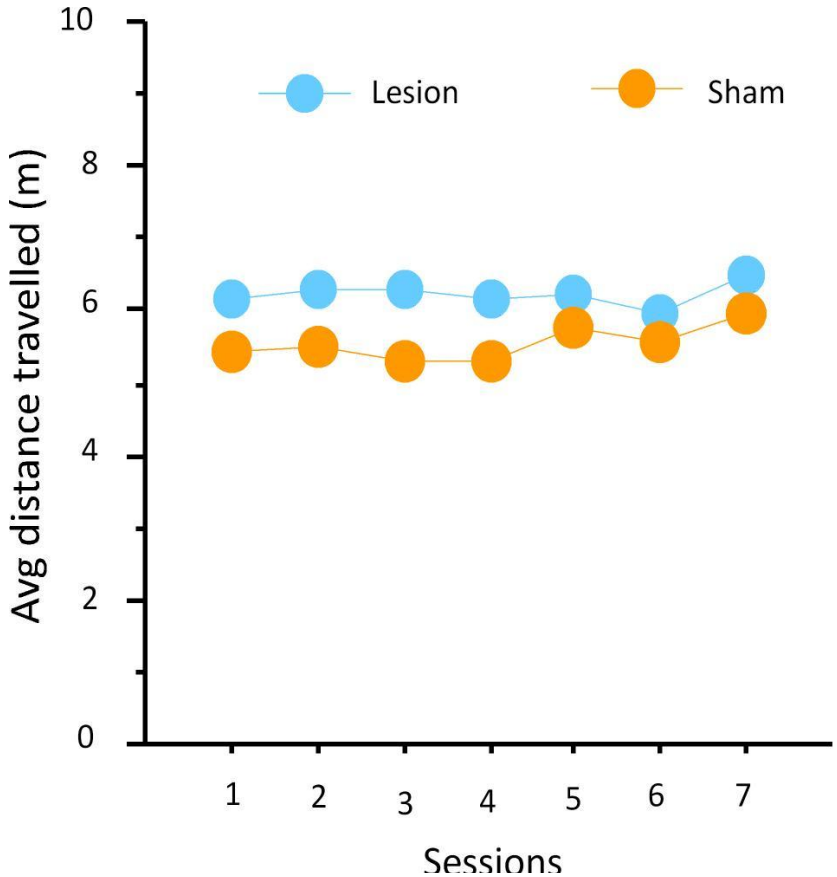

Figure 9. Average distance travelled by lesioned and sham rats in a 7 days treatment regimen of METH sensitization. The graph shows an effect of session and lesion.

4.4. Effects of lesion on METH-induced stereotypy

To determine whether receiving a chronic neurotoxic regimen of METH produced any stereotypic behaviour, animals were administered a dose of METH (2 mg/kg) for 7 days. Stereotyped behaviour was measured daily with a rating scale (Creese and Iversen, 1972). The rating was based on behavioural observation performed twice for 1 min, at 20 min and 50 min after the injection (2 mg/kg). The average of the 2 scores was considered as the overall session score and the raw scores converted to rank scores. 4 domains of stereotyped behavior were considered: repetition, frequency, duration and spatial distribution. There was no significant effect of lesion on repetition domain of stereotypic behavior ($F=2.028$, $df= 1$, $p= 0.1736$).

However, repetition alone as a measure of stereotypic behaviour was found to be significant (F= 19.892, df= 6, p= <0.0001). The interaction effect of repetition and lesion was also non-significant (F= 0.378, df= 6, p= 0.8912) [Figure 10].

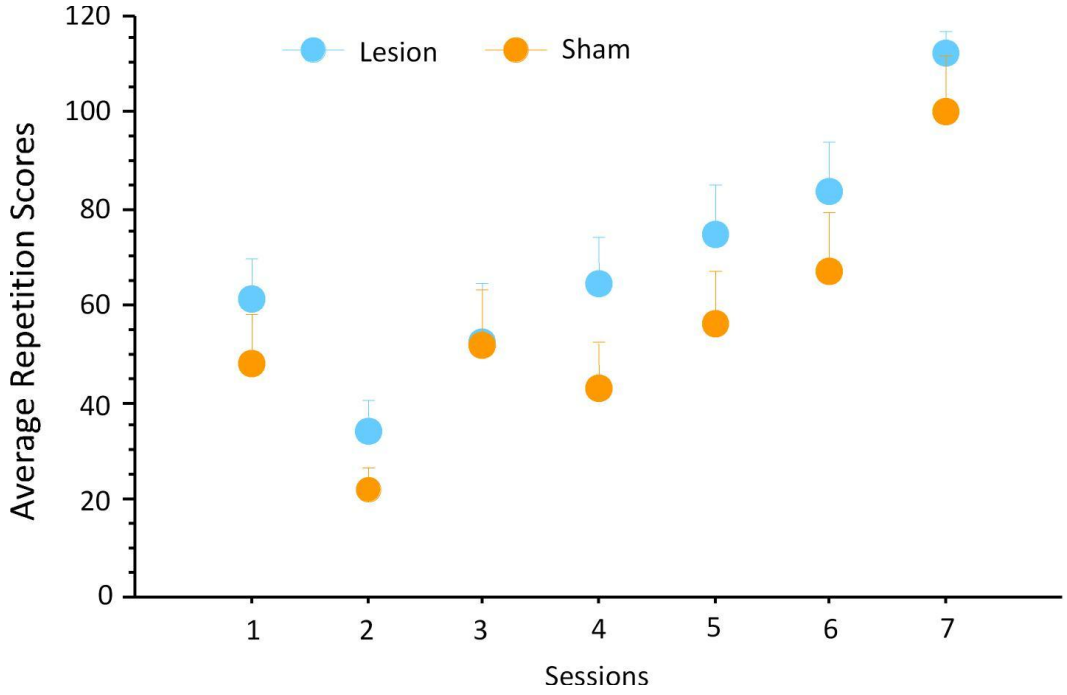

Figure 10. Average repetition scores of stereotypy measure in a 7 days acute METH treatment program. Data points represent means ± SEM.

The main effects of lesion and frequency as a measure of stereotyped behaviour were non-significant (F= 0.595, df= 6, p= 0.7332). The interaction effects of frequency and lesion was also found to be non-significant (0.595, df= 6, p= 0.7332) [Figure 11].

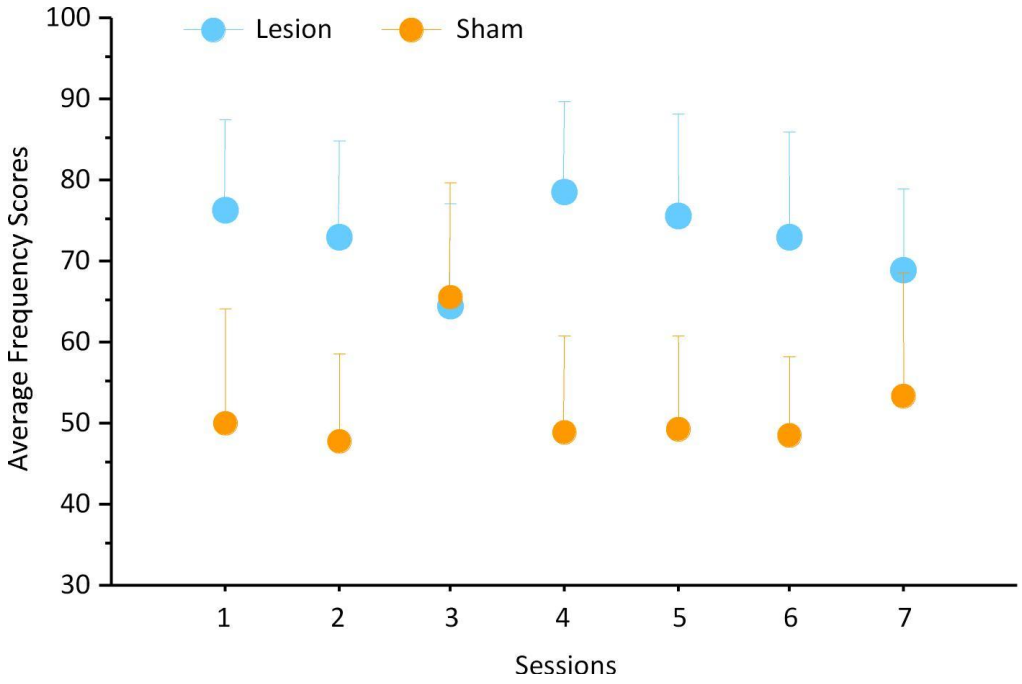

Figure 11.Average Frequency scores of stereotypy measure in a 7 days acute METH treatment program. Data points represent means ± SEM.

There was no significant effect of lesion on duration as a domain of stereotypic behavior (F= 3.039, df= 1, p= 0.1005). Also, the main effect of duration as a measure of stereotypic behaviour was not found to be significant (F= 0.58, df= 6, p= 0.9992). The interaction effect of duration and lesion was also non-significant (F= 0.252, df= 6, p= 0.9574) [Figure 12].

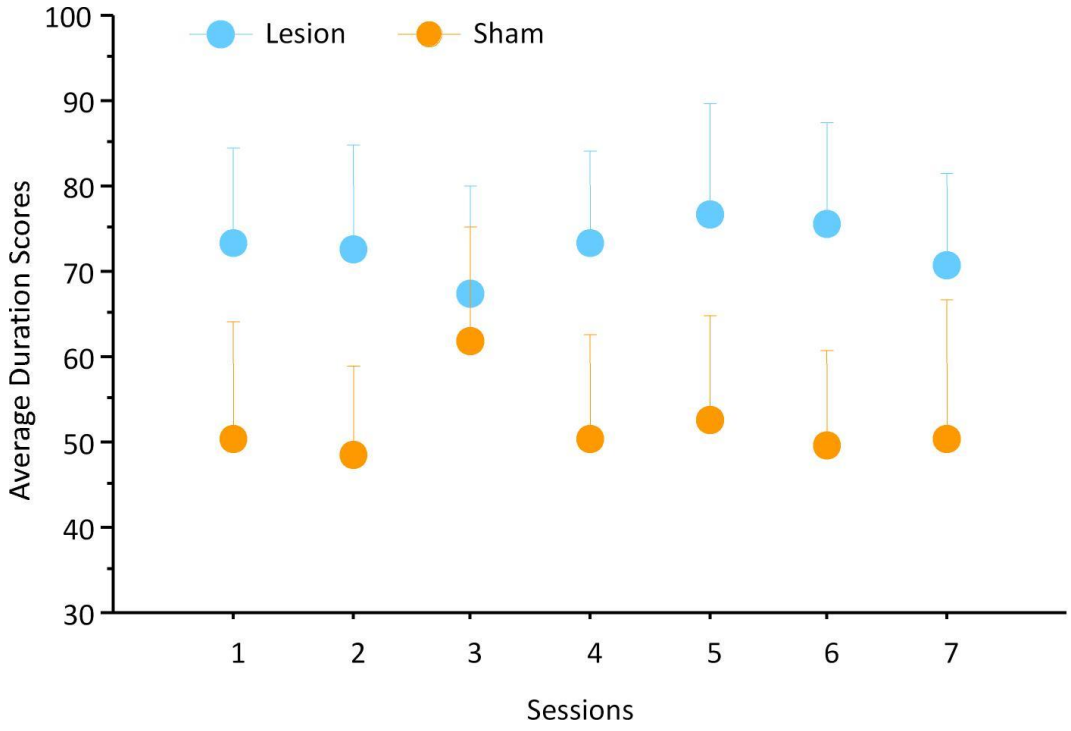

Figure 12. Average Duration scores of stereotypy measure in a 7 days acute METH treatment program. Data points represent means ± SEM.

The main effect of lesion on spatial distribution as a measure of stereotypic behavior (F= 1.238, df= 1, p= 0.2822) was non-significant along with the main effects of spatial distribution (F= 0.599, df= 6, p= 0.7305) and the interaction effects of lesion and spatial distribution (F= 0.265, df= 6, p= 0.9520) [Figure 13].

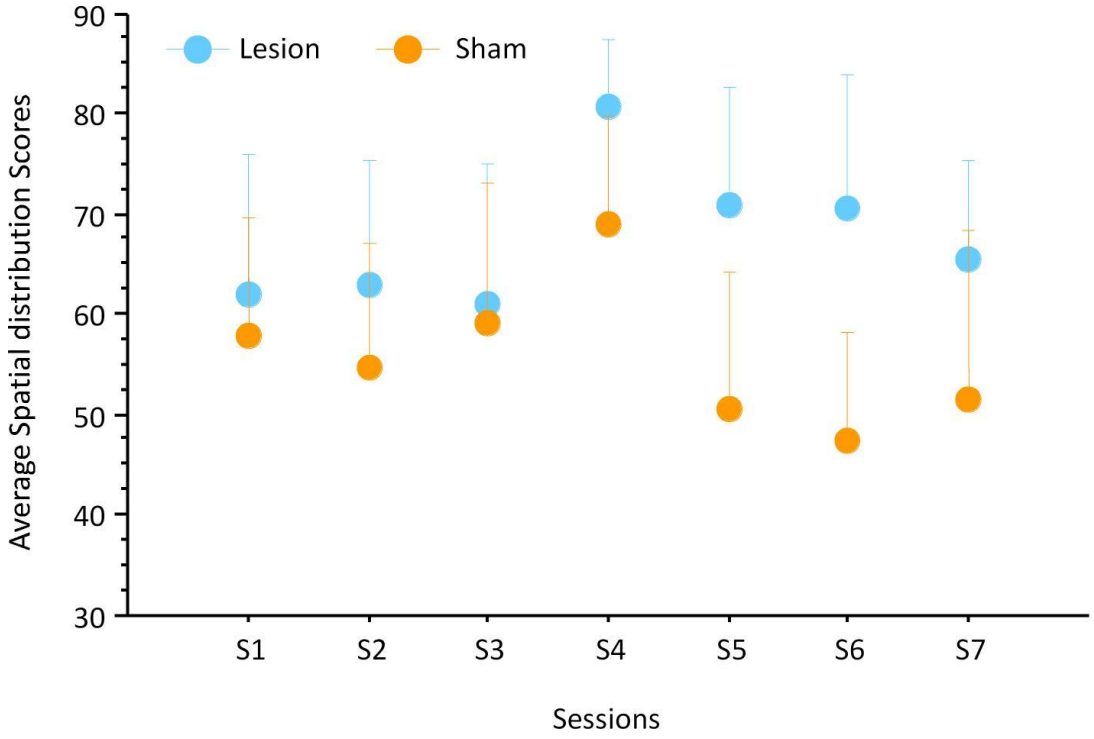

Figure 13. Average Spatial distribution scores of stereotypy measure in a 7 days chronic METH treatment program. Data points represent means ± SEM.

4.5. Effects of lesion on METH probe test

To examine the expression of long-term locomotor sensitization, animals were administered a subsequent low dose of METH (0.3 mg/kg). Increased METH-stimulated locomotion was found in rats previously sensitized to METH. Also, lesioned animals displayed significantly more hyperactive behaviour than the sham rats in the post probe phase (F= 6.480, df= 1, p= 0.0216) than in the pre-probe condition. The treatment effects of probe test and time was also found to be significant (F= 2.124, df= 23, p= 0.0021) [Figure 14].

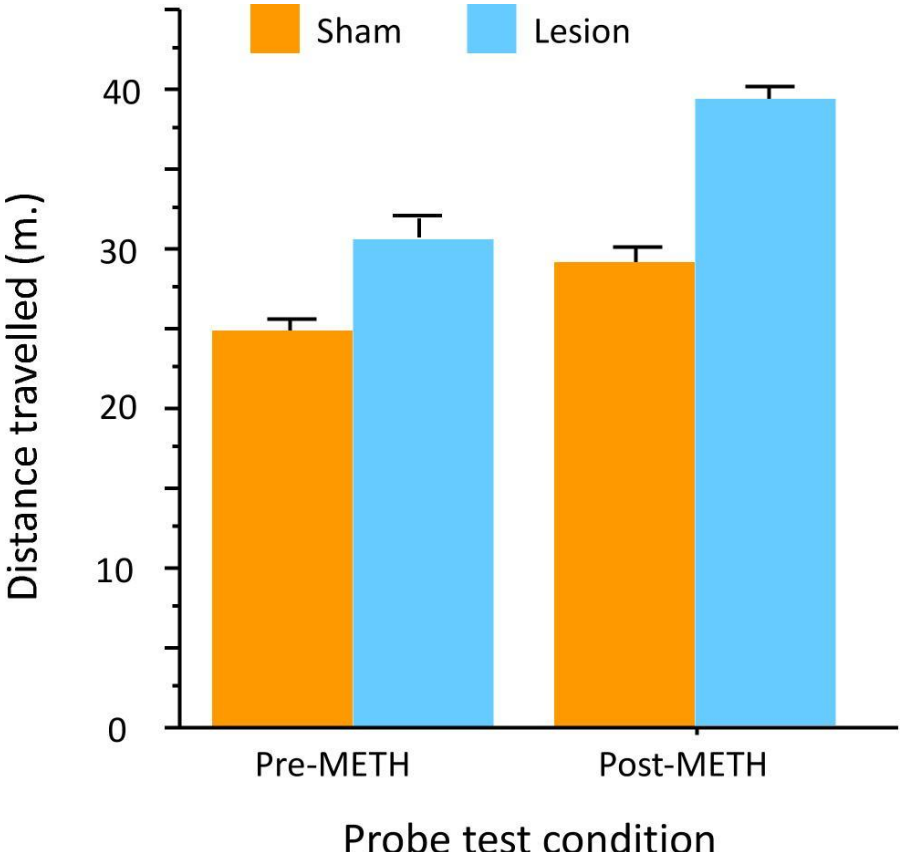

Figure 14. Locomotor activity recorded in terms of distance travelled by lesion and sham animals in the probe test condition. Bars represent means ± SEM.

4.6 Effects of lesion on working memory function

To determine whether receiving bilateral DDG neurotoxic infusions of colchicine produced any changes in working memory, animals were trained in a delayed alternation task in a T-maze. Analysis of the number of correct responses given by rats showed significantly less errors and more correct responses in the sham rats than in the lesioned rats. The main effect of lesion was found to be significant (F= 7.797, df= 1, p= 0.0130). However, the interaction effect of lesion and days was not significant (F= 0.531, df= 6, p= 0.7835) [Figure 15]. This suggests that the bilateral DDG lesions interfered with working memory.

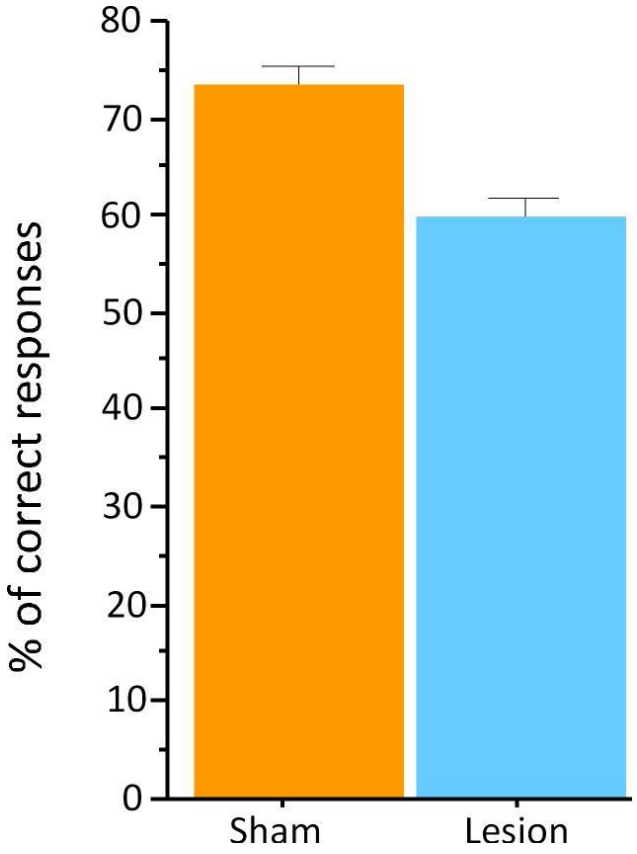

Figure 15. Percentage of correct responses given by sham and lesion rats in a delayed alternation task. Bars represent means ± SEM.

Discussion

The present research aimed to study the effects of bilateral DDG lesion on perseveration in a T-maze gambling task, METH-induced locomotor activity and stereotypy, sensitization to METH and also to study the bilateral DDG lesion effects on perseveration after administration of METH. The lesions targeted at DDG were more extensive than anticipated. The lesions damaged surrounding areas of the DDG, including removal of CA1 and medial subiculum in some cases. Hence, some effects may be due to damage to DG or to a combination of effects due to damage to structures surrounding the DG.

Perseverative behavior was increased in lesioned rats as compared to their sham counterparts. Also increased perseverative choices were increased in lesioned rats in the post- METH condition. Significantly, increased METH-induced hyperactive behavior and stereotypic behaviours were also observed in lesioned animals compared with controls. These findings indicate that the regions within the dorsal medial hippocampus (DMH) maintain inhibitory control over repetitive behaviours, including perseveration and stereotypies.

The first aim of the present research was to study the effects of bilateral DDG lesions on perseveration. As expected, lesions produced an effect on perseverative behaviour. The findings revealed a significant difference in perseverative behaviour in the lesioned and sham rats. Experimental rats repeatedly entered the same arm for food reward. By contrast, the sham rats could readily switch responses to attain their food reward. Furthermore, METH exposure significantly reduced the latency to make repeated and switched choices in both lesioned and sham rats. Also, there was a significant decrease in side bias in lesioned animals following METH exposure; however, lesion and sham animals did not differ prior to METH exposure; finally, sham animals did not show a significant change in side bias following METH exposure.

However, these positive findings could not be entirely attributed to DG function only, as the lesions encompassed areas beyond the DDG, including the CA1, CA3 subfields, the medial subiculum and parts of CA2.

Recurrent perseveration, the inappropriate repetition of previous specific responses or movements, is associated with dorsal striatal dysfunction (Luria 1965; Norman & Shallice 1986; Turner 1997). Frith & Done (1983) first demonstrated the correlation between recurrent perseveration and stereotypy in schizophrenia using a simple gambling task. In the present research, perseveration was quantified as the number of trials where the previous response was repeated. Whereas choices reflect the final output of the basal ganglial systems, the timing of choices, especially comparing repeated *versus* switched choices, can reveal underlying shifts in the mechanisms of response selection (Dallaire et al 2011; Garner et al 2003).Accordingly, we also quantified response latencies for both response types. Finally side bias (i.e., the overall chance of choosing one side or another) captures the sensitivity of animals to reward contingencies in general. Of particular relevance here, METH can shift animal's side biases towards more predictable or more immediate rewards (Perry et al 2008) – such 'delay discounting' measures are generally interpreted as a measure of limbic impulsivity. In this task, animals more sensitive to reward contingencies, animals preferring more predictable outcomes, or animals discounting risk would be expected to respond more to the bias correction procedure and show lower overall side biases.

One aim of the research was to measure locomotor activity and stereotypic behaviours after administration of METH. It was expected that METH exposure would produce increased locomotion and stereotypic behaviours. In support of the expectations, the findings demonstrated METH exposure significantly increased locomotor activity and stereotypic behaviour after chronic exposure to METH. METH is thought to increase brain levels of dopamine, norepinephrine and serotonin (Homer et al 2008). Research around the neurotoxicity of METH has suggested exposure influences the limbic system, including the hippocampus, grey matter and corpus callosum (Barr et al., 2006). It is proposed that adolescent exposure produces alterations in these brain regions and these persist into adulthood.

Chronic exposure to METH significantly increased stereotypic behaviours in rats. However such increased stereotypic behaviours were more prominent in lesioned rats than the sham counterparts. This finding implies that the interaction between the drug METH and damage to the hippocampal brain areas enhanced the manifestation of stereotypic behaviours. Rats administered a chronic dosing regimen of METH for a period of 7 days showed significantly more stereotypic behaviours, such as head rearing and sniffing, than shams. There was no significant effect for frequency, duration and spatial distribution, with a significant difference found in repetition. This finding implies that the lesioned animals did not show any significantly greater stereotyped behaviour in terms of frequency, duration and spatial distribution however, the experimental group significantly repeated more stereotyped behaviour than the shams.

One aim of the research was to study locomotor activity and stereotypy after acute exposure to METH and another aim was to study them after chronic exposure (sensitization) to METH.
METH induces monoamine depletions thought to contribute to cognitive and behavioural dysfunctions (Son, Kuhn & Keefe, 2012). METH is a psychomotor stimulant that increases locomotor activity when administered at low doses and elicits stereotypic behaviour when administered at higher doses (Kelly et al., 1975; Florin et al., 1994). Consistent with this idea, our present research findings confirm that rats who were administered METH at low doses showed increased locomotion and the same rats when administered METH at high doses displayed stereotypic behaviours. This might be due to dopaminergic transmission in the nucleus accumbens and the caudate nucleus which mediates METH-induced hyper-locomotion and stereotypy, respectively, as has been found in previous studies, namely Creese and Iversen, 1974; Kelly et al., 1975; Kelly and Iversen, 1975; Lucot et al., 1980.

In previous studies of laboratory animals, deficits associated with METH-induced neurotoxicity also include impairments in behavioural and cognitive flexibility (Izquierdo et al., 2010; Kosheleff et al., 2012; Pastuzyn et al., 2012; Son et al., 2011). In the present research, it was found that after administration of METH, rats showed behavioural inflexibility in terms of

stereotypic behaviours, including head rearing and sniffing. Impairments in cognitive flexibility, in terms of perseveration were also found in METH-treated rats.

Several behavioural effects are produced by reduced behavioural inhibition that accompanies drug-induced stereotypies. For example, METH enhances the rate at which behaviours are initiated and impairs performance in tasks where responses must be suppressed or slowed down (Evenden & Robbins 1983). Human patients with lesions involving the dorsal striatum show a general tendency to inappropriately repeat previous responses and movements: a phenomenon known as 'recurrent perseveration' (Luria 1965; Turner 1997). Deprivation-reared rats and primates also perform poorly in tasks sensitive to recurrent perseveration (Einon et al. 1975), such as extinction learning (Beauchamp & Gluck 1988; Jones et al. 1991). Schizophrenic patients often inappropriately repeat words, and this tendency correlates with their levels of stereotypy (Manschreck et al. 1981; Crider 1997). Both autistic and schizophrenic patients also show recurrent perseveration in several diagnostic tasks, and again, this tendency correlates with their stereotypy (Frith & Done 1983; Turner 1997). Thus, if METH-induced stereotypies similarly reflect changes in dorsal striatal function then they too should correlate with other symptoms of decreased inhibition, such as the inappropriate repetition of previous responses in specific experimental tasks. Since the dorsal striatal motor circuits of rodents are similar to those of mammals in terms of division into an indirect and direct pathway and connections with other motor areas (Medina & Reiner 1997; Reiner et al.1998), we predicted that the METH-induced stereotypies of rats would have similar correlates to those of mammals.

Behavioural studies have confirmed functional interactions between hippocampus and nucleus accumbens that manifest themselves as perturbations in locomotor activity (Wilkinson et al 1993). Thus, lesions of the hippocampus have been shown to enhance METH-induced locomotion, an effect dependent on the integrity of dopaminergic terminals within the nucleus accumbens (Whishaw and Mittleman 1991). Furthermore, some neurochemical data provides evidence of changes in dopamine turnover in nucleus accumbens in animals bearing hippocampal lesions (Lipska et al 1993).Clearly, the notion that the hippocampus can influence

motor behaviour by interacting with dopamine transmission in the nucleus accumbens is an attractive one, embracing the well documented role of meso-accumbens dopamine as a critical factor in determining motor functions (Pijnenburg et al 1976). Wilkinson et al (1993) have found that the hippocampus is involved in the control of locomotion and provides direct evidence that this influence is exerted, at least in part, via projections that modulate dopamine transmission in the nucleus accumbens.

Potentiated locomotor response to METH has been associated with an increased sensitivity of the dopaminergic system and used as a model of the positive symptoms of schizophrenia in rodents (Lecourtier et al 2010).The hippocampus, through the subiculum, modulates dopamine transmission and hippocampal or subicular lesions potentiate the locomotor response to METH (Lecourtier et al 2010). In our present research, lesions of the hippocampus and subiculum might be the causal factor behind increased METH-induced locomotor activity and stereotypic behaviour. Indirect evidence that hippocampal dysfunction results in a profound perturbation of the dopaminergic system come from studies showing that hippocampal lesions potentiate the locomotor response to METH (Coutureau et al 2000; Lipska et al 1992). As the locomotor response to METH is principally mediated by an increase of dopamine release in the nucleus accumbens (Pierce, Kalivas 1997), a potentiated locomotor response to METH is thought to result from a generalized hypersensitivity of the meso-accumbens dopamine system.

Interestingly, perseverative behaviour has been reported in chronic METH abusers. For example, Henry et al. (2011) reported that chronic METH abusers exhibited increased perseverative interactions with both novel and already-engaged objects in a novel open-field paradigm. Similarly, other studies have reported that METH-dependent individuals make more perseverative errors than controls in the Wisconsin Card Sorting Task and that the greater number of errors is associated with hypo-metabolism in the prefrontal cortex of those individuals (Chung et al., 2007; Kim et al., 2005, 2009). The present results in an animal model raise the possibility that such perseverative behaviour arises as a consequence of METH-induced neurotoxicity as lesioned rats show more perseveration in the post-METH condition.

The basis for the perseveration observed in individuals with a history of METH exposure is not yet clear; however, it has been suggested that corticostriatal circuitry is highly associated with perseveration (Graybiel, 1998, 2000; Haber and Calzavara, 2009). Previously, it has been reported that METH-induced neurotoxicity is associated with altered learning and memory processes subserved by corticostriatal circuitry (Pastuzyn et al., 2012; Son et al., 2011).

Recent studies that incorporate a sub-regional analysis of the hippocampus suggest a heterogeneous distribution of function in the DG, CA1 and CA3 subfields, with the DG being probably implicated in providing a primary, sparse representation of space early during learning and facilitating encoding of spatial elements in conjunction with CA3 (Rolls and Kesner, 2006; Rolls, 2007). In addition, the DG is possibly required to allow discrimination between similar contexts (McHugh et al., 2007), including those that generate conditioned affective responses through association with aversive and hedonic experiences (Hernandez-Rabaza et al., 2008). Last, the DG appears to be critically involved in working memory performance. Selective ablation or genetically-induced alterations of the DG severely disrupt spatial working memory but spare place learning and acquisition of simple object-place associations (Hernandez- Rabaza et al., 2007). In the present research, we have accordingly explored in rats the role of DG in working memory performance. We have found an impairment of working memory function after bilateral lesion of the DMH. This clearly implies that DG and adjoining areas in the DMH mediate spatial working memory function. It has been found that rats with hippocampal lesions are impaired in using environmental spatial cues to remember particular places (Cassaday and Rawlins, 1997; Jarrard, 1993; Kesner et al., 2004; O'Keefe and Nadel, 1978), to utilize spatial cues or bridge delays (Kesner et al., 2004; Kesner and Rolls, 2001; Rawlins, 1985), and to perform relational operations on remembered material (Eichenbaum, 1997).

According to Olton et al (1978) working memory contains information relevant to a given trial, and is context specific. Olton et al. (1979) proposed that the hippocampal system is involved in working memory. Rawlins (1985) proposed that the most critical impairment following

hippocampal damage was the ability to deal with temporal discontinuity between the events to be associated. According to this view, establishing an association between events that do not overlap depends on the maintenance of memory of the first event until the occurrence of the second, significant event; this ability would require the hippocampal formation. Wallenstein et al. (1998) have defended a similar proposal. In their view, the phenomenon can be seen as an intermediate-term memory store that bridges gaps (discontinuities) between stimuli that are to be associated. It is not surprising, according to this hypothesis, that impairments induced by hippocampal damage are most likely to occur in working memory tasks. Regarding the disruption of spatial learning following hippocampal damage, the construction of cognitive maps is assumed to require temporary memory storage for the maintenance of a wide range of cues so as to learn the (spatial) relations among them. In addition, dealing with spatial layouts requires time since the animals must explore the environment to gather information. Although these proposals disagree on the nature of the memory function to which hippocampal formation is related (either spatial working, or intermediate-term memory), they do share the common basic assumption that the integrity of the complete hippocampal circuit is crucial. According to this idea, the activities of the subcomponents of the hippocampal formation would be inter-related and, further, the interruption of the circuit at any specific point should produce similar behavioural outcomes in relation to either hippocampal damage as a whole or hippocampal disconnection. The hippocampal formation comprises a set of structures that are linked by well-defined trisynaptic (entorhinal cortex → DG → CA3 →CA1), disynaptic (entorhinal cortex → CA3 → CA1) and monosynaptic (entorhinal cortex → CA1) circuits. There have been attempts to assign specific roles to these circuits and to their different sub components. The DG cells are of special interest since they receive excitatory input from the entorhinal cortex via the perforant path, which activates pyramidal cells among the CA4 and CA3 pyramidal neurons, which over the Schaffer collaterals, contact CA1 pyramidal cells. The DG is thus in a position to control the flow of information within the hippocampus.

Implications of Research and scope for Future Research

Results of the present research have helped in developing a better understanding of the role of the DG and its adjoining areas comprising of the CA1, CA3 subfields and subiculum. It can be implied from the study findings that alterations in the hippocampal formation could be responsible, at least in part, for repetitive behaviour patterns observed in humans. Further experiments could be done in areas of learning and higher level cognitive functions to understand the overall nature of repetitive behaviours. In human neuropsychiatric disorders, like schizophrenia and autism, ARB forms part of the core symptoms. Therefore, future studies in this area could help in understanding the biological basis for ARB. And once the causal factor of the repetitiveness is found, it would lead to effective therapeutic treatments for the alleviation of symptoms.

The results from this research have substantial implications for society given the impact METH exposure may have in humans. A recent publication (The New Zealand Herald, 2012) reported that New Zealand has the highest rate of METH use in the world. The use of drugs like METH was the highest in the world, with 2.8 per cent of people having used it in New Zealand in 2011.These results indicate that increasing the understanding of how METH affects behavior may correct misguided assumptions about its use and have a positive influence on how adults choose to use and abuse drugs.

Conclusions

In conclusion, the present research indicates that areas of the DMH play a vital role in perseveration and increased drug-induced motor behavior in adult rats.

In addition, rats with lesions of the DMH were hypersensitive to METH after chronic exposure to the drug.

Moreover, rats with DMH lesions were impaired in working memory performance.

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